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Review

Atrial cardiomyocyte calcium signalling ☆

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ABSTRACT

Whereas Ca^{2+} signalling in ventricular cardiomyocytes is well described, much less is known regarding the Ca^{2+} signals within atrial cells. This is surprising given that atrial cardiomyocytes make an important contribution to the refilling of ventricles with blood, which enhances the subsequent ejection of blood from the heart. The dependence of cardiac function on the contribution of atria becomes increasingly important with age and exercise. Disruption of the rhythmic beating of atrial cardiomyocytes can lead to life-threatening conditions such as atrial fibrillation. Atrial and ventricular myocytes have many structural and functional similarities. However, one key structural difference, the lack of transverse tubules ("T-tubules") in atrial myocytes, make these two cell types display vastly different calcium patterns in response to electrical excitation. The lack of T-tubules in atrial myocytes means that depolarisation provokes calcium signals that originate around the periphery of the cells. Under resting conditions, such Ca^{2+} signals do not propagate towards the centre of the atrial cells and so do not fully engage the contractile machinery. Consequently, contraction of atrial myocytes under resting conditions is modest. However, when atrial myocytes are stimulated with a positive inotropic agonist, such as isoproterenol, the peripheral Ca^{2+} signals trigger a global wave of Ca^{2+} that propagates in a centripetal manner into the cells. Enhanced centripetal movement of Ca^{2+} in atrial myocytes leads to increased contraction and a more substantial contribution to blood pumping. This article is part of a Special Issue entitled: 11th European Symposium on Calcium.

1. Why are atrial myocytes important?

In a typical human lifetime, the heart beats over 2 billion times with the same repeated chain of events—the "cardiac cycle." The events can be divided into two key stages—diastole and systole. During the diastolic stage, the ventricular myocytes are relaxed. The systolic period refers to the contraction and consequent ejection of blood from the ventricles to the pulmonary artery or aorta. Atrial contraction and relaxation (the "P wave" in a normal electrocardiogram) occurs just before ventricular contraction.

The cardiac cycle is initiated by the sinoatrial node. This is a specialised group of non-contractile cardiac myocytes located within the right atrium that generate repetitive action potentials. From the sinoatrial node, an action potential spreads over both atria causing them to contract and push blood into the ventricles. Eventually, the action potential reaches the atrioventricular node, which temporally filters and relays the signal to the ventricles via specialised conduction tissue including the Purkinje fibres. The ventricular chambers are larger and stronger than their atrial counterparts, and are responsible for propelling blood to the lungs and body.

Although atrial contraction is not as substantial as that in the ventricles and does not generate the same force, atria can enhance the amount of blood that loads into the ventricles prior to systole. When a person is at rest, the contribution of atria to the filling of ventricles with blood is relatively low. Indeed, the majority of ventricular refilling occurs due the venous return of blood to the heart and ventricular relaxation. However, during periods of increased activity and hemodynamic demand, such as during exercise or stress, then atrial contraction can account for ~20–30% of the volume of blood entering the ventricles. The contribution of atria to ventricular refilling is sometimes called "atrial kick," and it can make a significant difference to the performance of the heart [1].

The most common form of cardiac dysrhythmia in humans is known as "atrial fibrillation." This pathology arises when electrical impulses do not solely arise from the sinoatrial node, but instead spontaneously occur with high frequency from sites around the atria (~350 discharges per minute compared to the normal sinoatrial rhythm of 60–80 beats per minute) [2]. Due to the irregular electrical discharges that occur during atrial fibrillation, the atria do not display coordinated contractions required to propel blood into the ventricles. Consequently, atrial kick is missing, and the blood pumping capacity of the heart can be reduced by a third [3]. Given that ventricles can refill substantially without atrial participation, atrial fibrillation is typically not immediately life threatening. However, thromboembolism caused by stagnation

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of blood flow is a significant complication arising from atrial fibrillation. With respect to human health, it is established that the incidence of atrial fibrillation increases with age, and ~15% of strokes occur in people with atrial fibrillation (see http://www.bhsoc.org/bhf_factfiles/bhs_factfile_dec_2000.pdf). It is therefore clear that co-ordinated atrial function is very important. Substantial evidence points to dysregulation of Ca^{2+} signalling as being a causal factor in genesis and maintenance of atrial fibrillation [4–8]. Heart failure is a progressive pathological change to the function of the heart caused by a chronic impairment in cardiovascular function, and if not managed it can be fatal. Heart failure is a common cause of atrial fibrillation, and it is evident that atrial myocytes phenotypically remodel during the disease such that they have reduced contraction, and a greater propensity to show spontaneous Ca^{2+} signals [5]. During the remodelling, atrial myocytes alter the expression of key proteins involved in Ca^{2+} homeostasis, storage and signal generation. Such changes unfortunately appear to reinforce the incidence of fibrillation [8].

2. Ca^{2+} and cardiac excitation–contraction coupling

As the action potential emanating from the sinoatrial node sweeps over the heart, it causes depolarisation of the myocytes, and consequently causes them to contract (a process known as excitation–contraction coupling; EC coupling) [9]. Ca^{2+} is the link between myocyte depolarisation and contraction. Ca^{2+} binds to troponin C within a complex of proteins that are associated with the thin (actin) filaments in myocytes. The binding of Ca^{2+} to troponin C displaces tropomyosin, and allows engagement of actin and myosin filaments. These myofilaments slide past each other and contract the cell. The simultaneous contraction of many cells within the atrial and ventricular walls generates sufficient force to propel blood around the heart, lungs and body.

Depolarisation of myocytes by the action potential causes voltage-operated Ca^{2+} channels (VOCCs) to open. These open channels permit Ca^{2+} to flow across the sarcolemma and into a narrow (~10 nm) cytosolic cleft (often referred to as the "dyadic junction") [10], which is formed by the juxtaposition of the sarcolemma and the membrane of the internal Ca^{2+} store (the sarcoplasmic reticulum; SR) (Fig. 1). The SR is studded with Ca^{2+} release channels known as ryanodine receptors (RyRs), which are activated by Ca^{2+} itself in a process known as Ca^{2+}-induced Ca^{2+} release (CICR) [11–13]. The density of RyRs is particularly high at the clefts, where the SR and sarcolemma are in close apposition, and they are therefore located directly opposite VOCCs. The Ca^{2+} that enters through VOCCs rapidly encounters RyRs and causes them to open. The release of Ca^{2+} sequestered within the SR greatly amplifies the original Ca^{2+} rise through the VOCCs. The dyadic junction is too small for visualisation with conventional imaging technologies; however, mathematical models have predicted that the Ca^{2+} concentration within this tiny volume may exceed several millimolar in the centre of the cleft [14,15].

The activation of RyR clusters via CICR within the dyadic junction leads to the generation of microscopic (diameter ~2–5 μm; lifetime ~100 ms) Ca^{2+} release events known as "Ca^{2+} sparks" [16,17]. Exactly how many RyRs participate in a Ca^{2+} spark is still debated. Estimates have suggested that Ca^{2+} sparks represent the activation of ~15 RyRs [18], although the variability in their time course and amplitude suggests that the number and/or cooperation between channels can vary [19,20]. The Ca^{2+} entry signal that activates the RyR cluster probably arises from a smaller group of closely apposed VOCCs [21]. Depending on the condition of the myocyte, Ca^{2+} sparks can represent an amplification (commonly also called "gain") of ~10-fold or more over the Ca^{2+} flux that originally entered through the VOCCs. Eventually, Ca^{2+} ions diffuse out of the cleft and trigger contraction by binding to troponin C within the myofibrils. Ca^{2+} recovers back to resting (diastolic) levels due to sequestration in the SR by an ATP-dependent enzyme known as SERCA (sarcoendoplasmic reticulum Ca^{2+} ATPase), and transports across the sarcolemma via the action of a Na^+/Ca^{2+} exchanger (NCX). Myocytes also express sarcolemmal Ca^{2+} ATPases (PMCAs). These enzymes are generally believed to have a minor effect on Ca^{2+} extrusion [22], but they may have other isoform-specific signalling roles [23].

3. Ventricular myocyte EC-coupling

Since the ventricles contribute most to blood pumping, and disturbances in their rhythmicity can be immediately life-threatening, Ca^{2+} signalling has been most extensively studied in ventricular myocytes. Depolarisation of mammalian ventricular myocytes causes homogenous whole-cell Ca^{2+} signals that activate myofilaments throughout the volume of the cells [17,24,25]. Such global Ca^{2+} signals arise from the spatial summation of Ca^{2+} ions released by thousands of simultaneously-activated Ca^{2+} spark sites. The critical ultrastructural aspect of mammalian ventricular myocytes that promotes global Ca^{2+} transients is the presence of T-tubules [26]. These inward projections of the sarcolemma arise at each of the Z-lines within cardiac muscle, and have a regular spacing (~1.8 μm) [27,28] (Figs. 1 and 2). T-tubules vary in diameter (20–400 nm) and can have highly-branched morphologies, with both transverse and longitudinal elements [29]. Despite their narrow diameter, it is estimated that T-tubules can account for ~50% of the sarcolemmal membrane [30].

Key proteins involved in the initiation and eventual recovery of Ca^{2+} signals are located along the T-tubules, with lesser amounts on the peripheral surface membrane [26]. With respect to EC-coupling, T-tubules bring VOCCs and RyRs into close proximity to form dyadic junctions within the whole volume of a ventricular myocyte. Indeed, immunostaining ventricular myocytes for VOCCs and RyRs reveals that these proteins spatially overlap throughout the cells [25,31–33] (Fig. 2). Essentially, T-tubules relay action potentials to VOCCs at dyadic junctions throughout the cell. Consequently, when a ventricular myocyte is depolarised, thousands of Ca^{2+} sparks are simultaneously triggered and a homogenous Ca^{2+} rise ensues [25,34]. Imaging ventricular myocytes at high speed during the up-stroke of a response reveals that Ca^{2+} signals originate with a striated appearance (due to discrete release at dyadic junctions) [35,36]. The rapid diffusion of Ca^{2+} from the release sites produces the subsequent homogenous global Ca^{2+} signal within tens of milliseconds [37]. The loss of T-tubules, as occurs in disease (see below), leads to clusters of RyRs that are "orphaned" in the sense that they are no longer within a functional dyad and are not activated by CICR from nearby VOCCs during EC-coupling. Imaging of myocytes with orphaned RyRs demonstrates spatial inhomogeneities within the Ca^{2+} transients. Such inhomogeneities cause myocytes to contract with less force [29].

4. Ca^{2+} signals during atrial myocyte EC-coupling

It is likely that all electrically active or contractile cell types within mammalian hearts possess some degree of T-tubule network, but that the degree of tubulation can vary between regions and species. For example, the atrial myocytes of rats, and of some other small mammals, have a more rudimentary transverse-axial tubular network that is quite different from that seen in their ventricular cells [10,25,28,38,39]. It has been suggested that in place of the T-tubules these atrial cells have prominent SR elements, termed "Z-tubules" (Fig. 1) [40]. Just like T-tubules, these structures are perpendicular to the long axis of the cells and are also spaced at ~1.8 μm intervals. Therefore, atrial cells also contain transversely-oriented tubules, but they are sometimes formed from internal SR membrane and not the sarcolemma.

The distribution of RyRs within atrial myocytes is similar to that observed in ventricular cells; the majority of RyRs are observed within transverse striations corresponding to the positions of the Z-tubules

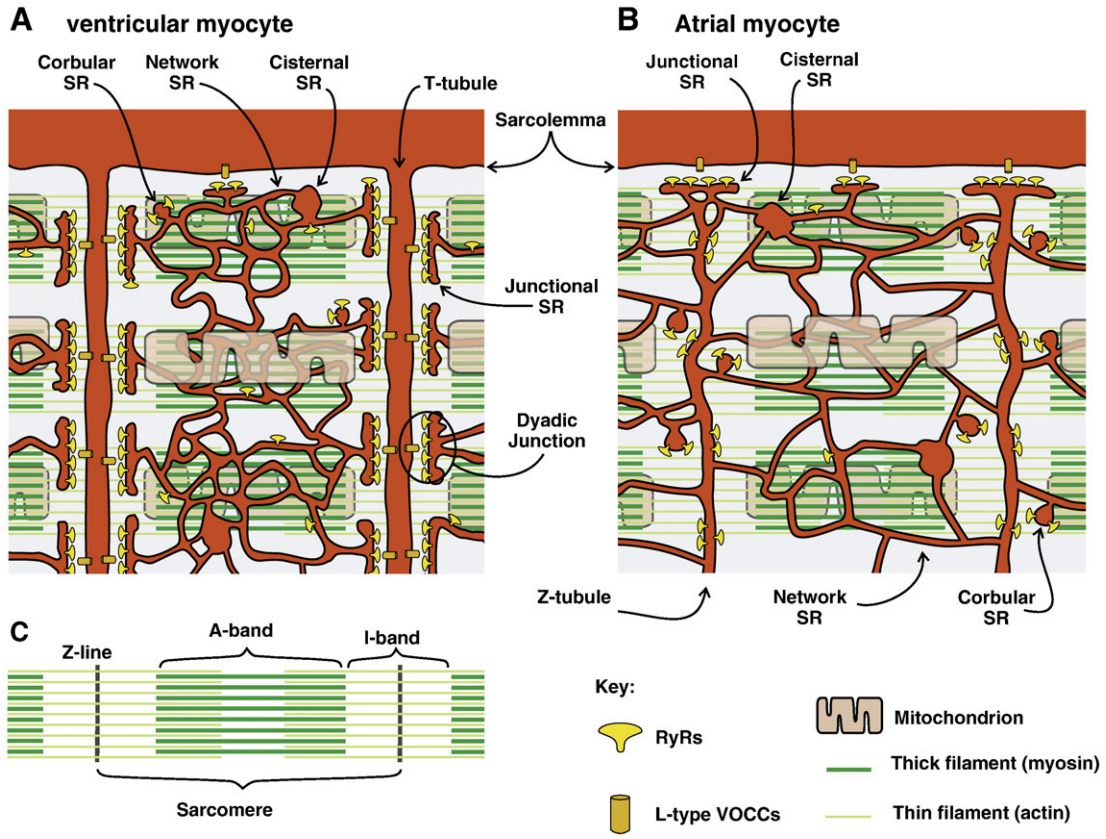

Fig. 1. Structure of ventricular and atrial cardiac myoctes. Panels *A* and *B* are cartoon depiction of portions, a ventricular and an atrial myocyte respectively. They illustrate the relative positioning of some of the key elements involved in EC-coupling. The network SR elements wrap around both the myofibrils and mitochondria. For additional representations of these cells see Refs. [40,122,123]. Panel *C* shows the topology of a myofibril relative to the ventricular myocyte section above it in *A*. The ventricular diagram depicts discrete junctional couplings directly with the peripheral sarcolemma in the middle of the A-band. These couplings have been clearly visualised in some studies [32], but are not always evident (Fig. 3Aii). Similarly, with atrial cells, it appears that junctional couplings exist between those at the Z-lines (Fig. 3Bii) [32]. Reproduced with permission from Ref. [27].

(Fig. 2B). However, there is one critical difference between atrial and ventricular myocytes; the expression of RyR clusters around the periphery of the atrial cells [41–43]. This is presented clearly in Fig. 3, which depicts the location of α-actinin (an actin-binding protein found at Z-lines; Fig. 3Ai–Ci), RyRs (Fig. 3Aii–Cii) and the sarcolemmal Na^+/Ca^{2+} exchanger (Fig. 3Aiii–Ciii) in a ventricular, atrial and neonatal cardiac cell. The striated appearance of the cells is evident from the α-actinin immunostaining (although the neonatal cell is not fully differentiated). RyR immunostaining overlaps with that of the α-actinin staining in all three cell types (data not shown). However, it is evident

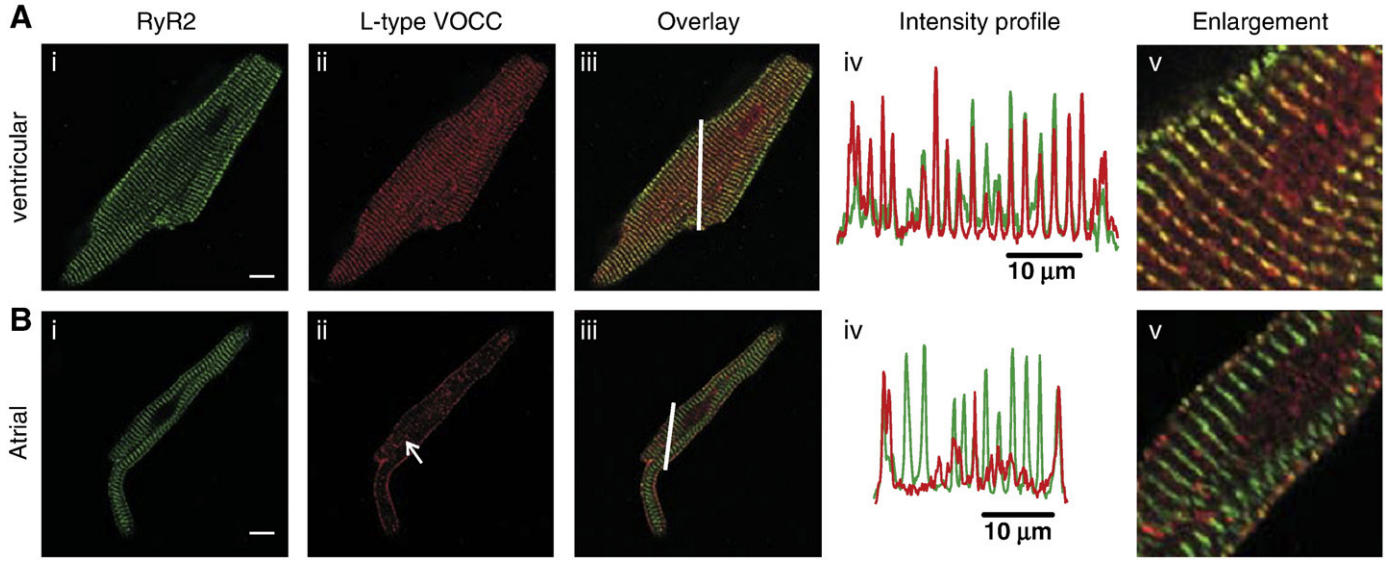

Fig. 2. Localisation of RyRs and L-type VOCCs in ventricular and atrial myocytes. The images in panels A and B depict single ventricular (A) or atrial (B) myocytes immunostained for type 2 RyRs or L-type VOCCs. The intensity profiles (Aiv and Biv) were obtained by measuring the intensity of fluorescent antibody labelling across the cellular regions depicted by the dashed white lines in the overlay images. The horizontal white bars in the cell images represent 10 μm. The data are representative of >50 cells for each condition, sampled from at least three independent preparations. Reproduced with permission from Ref. [25].

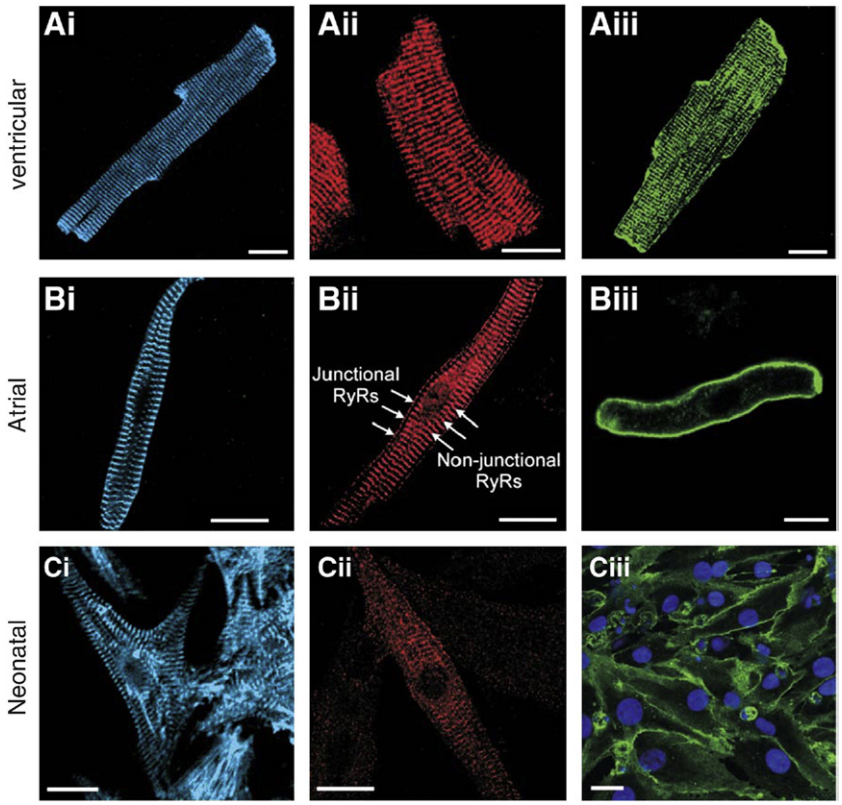

Fig. 3. Structure of ventricular, atrial and neonatal rat cardiac myocytes. Panels *A*, *B* and *C* depict immunostained adult ventricular, adult atrial and neonatal ventricular myocytes respectively. The left-hand panels (*Ai*, *Bi* and *Ci*) show cells immunostained with a polyclonal antibody raised against α-actinin (Sigma, UK). The middle panels (*Aii*, *Bii* and *Cii*) display cells immunostained with a polyclonal antibody raised against type 2 RyRs (a kind gift from Professor V. Sorrentino, San Raffaele Scientific Institute, Siena, Italy). The right-hand panels (*Aiii*, *Biii* and *Ciii*) depict cells immunostained with a monoclonal antibody raised against the type 1 sodium/calcium exchanger (R3F1; a kind gift from Professor K. Philipson, UCLA, California). The white bar in each panel depicts a spatial scale of 20 μm. To visualise the NCX staining in the neonatal myocytes, a field of confluent cells is depicted (*Ciii*) as this provided the best contrast in these flat cells. The neonatal myocyte nuclei were visualised using DAPI staining. Specific immunostaining was visualised using Alex-405, -488 or -568-conjugated secondary antibodies. The α-actinin staining shows the precise striated structure of the fully differentiated adult ventricular and atrial cells (*Ai* and *Bi*). The neonatal cells showed some α-actinin striation (*Ci*), but they did not have the same degree of organisation as in their adult counterparts. RyRs have a regular transverse striated pattern in adult cells (*Aii* and *Bii*). Note the two distinct populations of RyRs in adult atrial cells. In addition to the transverse "non-junctional" RyRs, atrial cells expressed a ring of "junctional" RyRs (*Bii*). The latter are responsible for the initiation of EC-coupling around the circumference of the cell. In the neonatal myocytes (*Ci*), the degree of RyR expression is lower and less organised than in adult cells. With both atrial (*Biii*) and neonatal cells (*Ciii*), the NCX staining is prominent only around the circumference of the cells. Whereas, with ventricular myocytes that express T-tubules, the NCX protein was evident on the sarcolemma and at the Z-lines deep inside the cells (*Aiii*). Reproduced with permission from Ref. [27].

that there is a discontinuity in the distribution of RyRs in atrial myocytes. RyR immunostaining is evident around the periphery of the cell beneath the sarcolemma (marked as "junctional RyRs," with a discernable gap in expression between them and the "non-junctional RyRs" (Fig. 3Bii). For the mature ventricular myocyte, the distribution of RyRs also correlates with that of the Na^+/Ca^{2+} exchanger (Fig. 3Aiii) on the T-tubules. However, in atrial and neonatal ventricular myocytes, which lack substantial T-tubules, RyR and Na^+/Ca^{2+} exchanger expression overlaps only at the cells' periphery.

Atrial myocytes therefore possess two populations of RyRs. One minor group (junctional RyRs) sit just beneath the sarcolemma. The other channels (non-junctional RyRs) are deeper inside the cell and constitute the bulk of the RyR population. Although the junctional RyRs represent a small fraction of the total number of RyRs, they are crucially important in atrial EC-coupling, as it is these junctional RyRs that are responsible for initiating CICR. Due to the lack of T-tubules, the sarcolemma does not regularly protrude into atrial cells. Therefore, the localisation of VOCCs is entirely different in ventricular and atrial myocytes; VOCCs are expressed and functional around the periphery of atrial myocytes [31]. As a consequence, the close apposition of RyRs and VOCCs that is necessary for triggering EC-coupling occurs solely in the immediate subsarcolemmal space at the cells' edges (Fig. 2B).

The differences in ultrastructure (i.e. T-tubules, VOCC location, and RyR distribution) between atrial and ventricular myocytes impact significantly on the spatial pattern of Ca^{2+} signalling in these two cell types. As described above, ventricular myocytes display homogenous responses, which arise from the simultaneous recruitment of Ca^{2+} sparks throughout a cell. However, in atrial myocytes, EC-coupling is initiated around the periphery of the cells, because this is the only place where the VOCCs and junctional RyRs come together to form dyadic junctions. Rapid imaging of Ca^{2+} responses in atrial myocytes has demonstrated that Ca^{2+} sparks are rapidly triggered around the periphery of the cell [41,44]. Subsequently, the Ca^{2+} signal emanating from the spark sites appears to spread laterally, eventually yielding a contiguous Ca^{2+} signal that is solely located around the edge of the cells (Fig. 4A and B). Any further development of the Ca^{2+} signal depends on the status of the myocytes, with factors such as the Ca^{2+} content of the SR and the presence of "positive inotropes" (agents that increase the force of heart contraction) determining whether the peripheral Ca^{2+} signal will pass further into the centre of the cells [31]. Key positive inotropes are the catecholamines epinephrine and norepinephrine, which can reach the heart via the circulation or local release from sympathetic nerve terminals [9]. Other positive inotropes include endothelin, angiotensin and growth factors such as insulin-like growth factor.

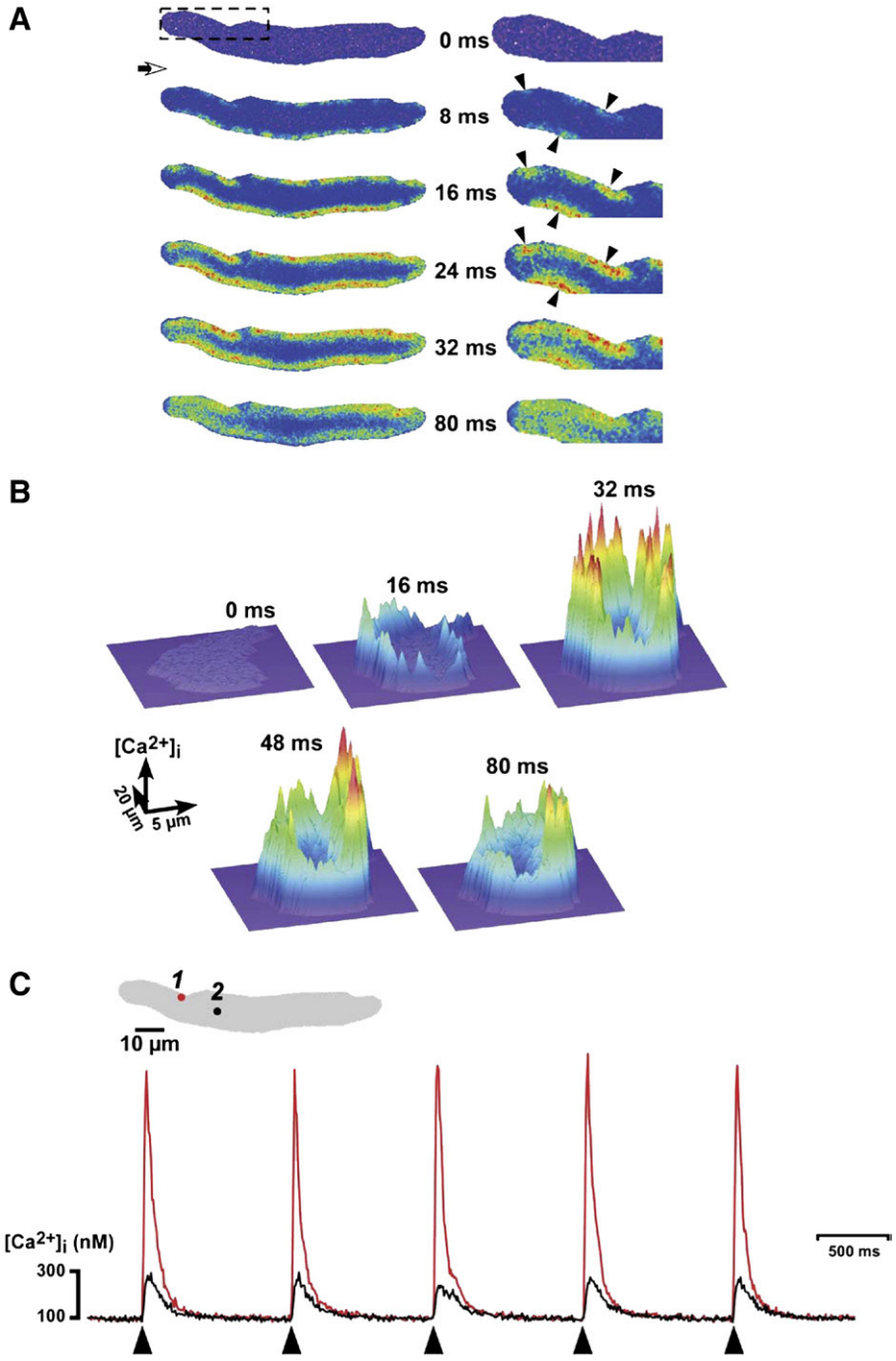

Fig. 4. Spatially heterogeneous Ca^{2+} signalling during atrial myocyte EC-coupling. Panel *A* shows the development of a Ca^{2+} signal following electrical depolarisation in a single atrial myocyte. The left-hand montage shows pseudocolour-coded images of the entire cell. To indicate the initiation of discrete Ca^{2+} sparks (designated by the arrowheads) and their lateral spreading, the right-hand column of images depicts part of the cell (the region bounded by the dashed box) at a higher magnification. The time at which the electrical pulse was applied is shown by the open arrow. Three obvious Ca^{2+} spark sites are marked by black arrowheads. The surface plots in panel *B* depict the spatial and temporal development of a Ca^{2+} signal from a different atrial cell. The Ca^{2+} concentration is encoded by both the colour and in the height of the surface. A clear peripheral ring of Ca^{2+} is evident with a steep "valley" of low Ca^{2+} concentration in the centre of the cell. Panel *C* illustrates the consistency of the spatial gradient of Ca^{2+} signalling in atrial myocytes during electrical pacing. The traces show the Ca^{2+} concentration at the two cellular regions depicted on the inset cell image. The peripheral region (red circle and red trace) displayed a large Ca^{2+} rise with each depolarisation (denoted by the black arrowheads). Whereas, the central region had a lower response (black circle and black trace). Reproduced with permission from Ref. [27].

In (non-tubulated) rat atrial myocytes paced to steady state in the absence of positive inotropes, each Ca^{2+} response is similar to that described above—a flurry of peripheral Ca^{2+} sparks followed by lateral diffusion of the Ca^{2+} ions, ultimately producing a subsarcolemmal Ca^{2+} signal that does not propagate significantly into the centre of the cells (Fig. 4C) [31,41]. Under these conditions, there is a substantial Ca^{2+} gradient from the edge of the cells to the centre. Indeed, the peripheral Ca^{2+} signal can reach a peak of ~1 µM, whereas centre of the cell barely shows an increase over the normal prestimulated Ca^{2+} level of ~100 nM. This spatially heterogeneous pattern is even maintained while the Ca^{2+} transients recover back to their pre-depolarisation (diastolic) condition (Fig. 4C). Numerous studies using atrial myocytes from a range of species, including rat [35,41,45], guinea pig [46,47], cat [44,48,49] and human [43], concur in that atrial EC-coupling arises from the activation of peripheral Ca^{2+} release sites.

5. Multiple mechanisms cause the peripheral restriction of Ca^{2+} signals in atrial myocytes

Under basal stimulation conditions, as described above, the Ca^{2+} elevation evoked by depolarisation of an atrial myocyte is largely restricted to the periphery of the cells, and contraction is relatively modest. Assuming typical atrial cell dimensions, <20% of the volume of a myocyte would be occupied by such a peripheral Ca^{2+} signal. The majority of myofilaments that need to bind Ca^{2+} in order to cause contraction are located deeper inside the cell. The Ca^{2+} signal must therefore penetrate further inside atrial myocytes, and occupy a more substantial proportion of their volume, to trigger greater contraction.

Since the junctional RyRs are closest to the VOCCs, they respond under all stimulation conditions. The non-junctional RyRs constitute a 3-dimensional matrix of Ca^{2+} release clusters throughout the centre of the myocytes' volumes. The spacing between the clusters of non-junctional RyRs is ~1 μm in the transverse and ~1.8 μm in the longitudinal directions. It would therefore be expected that the non-junctional RyRs would sense the subsarcolemmal Ca^{2+} sparks triggered at the beginning of EC-coupling, and subsequently relay the Ca^{2+} signal deeper into the myocyte via successive rounds of CICR. Intracellular Ca^{2+} waves have been shown to propagate in such a saltatoric manner between clusters of channels [44,50–52]. However, in the case of atrial myocytes, this centripetal movement of Ca^{2+} clearly does not occur when EC-coupling is initiated under basal conditions.

There are several plausible explanations why EC-coupling does not activate centripetal Ca^{2+} waves under basal conditions, and they all relate to the effectiveness of the subsarcolemmal Ca^{2+} signal in triggering CICR from the non-junctional RyRs. Essentially, under some conditions, the subsarcolemmal Ca^{2+} signal is not a sufficient trigger for a saltatoric Ca^{2+} wave, or the non-junctional RyRs are not sufficiently sensitive to respond to the trigger signal.

One potential interference in the triggering of centripetal Ca^{2+} waves is the ~2 μm gap that separates the junctional RyRs and non-junctional RyRs in atrial cells [31,32,41,42,44] (Figs. 2 and 3). Ca^{2+} can rapidly diffuse in aqueous solution, but within the cytoplasm of cells its movement is dramatically slowed by a substantial number of binding sites, such that the concentration of Ca^{2+} declines dramatically with distance from a point source [53]. It is therefore plausible that the ~2 μm gap in RyR distribution between junctional and non-junctional RyRs allows for a substantial decline in the profile of the subsarcolemmal Ca^{2+} signal so that there is an insufficient trigger to activate further CICR. Beyond the gap, the non-junctional RyR clusters are spaced <1 μm apart (in the transverse direction; they are ~1.8 μm apart along the longitudinal axis) [32], so that Ca^{2+} wave propagation is more likely once the gap has been overcome.

In addition to the diffusion gap between junctional and non-junctional RyR clusters, the movement of Ca^{2+} ions from the subsarcolemmal region is significantly hindered by rapid Ca^{2+} transport into organelles. In particular, mitochondria and SERCA can provide a powerful barrier against cellular Ca^{2+} rises [54–56]. Electron microscopy and immunofluorescence studies have confirmed that mitochondria and SERCAs are closely aligned with the junctional RyRs [44], and we have demonstrated that they restrict the movement of Ca^{2+} in atrial myocytes [31]. Inhibition of either mitochondrial Ca^{2+} uptake or SERCA activity enhances the ability of subsarcolemmal Ca^{2+} signals to trigger centripetal Ca^{2+} waves in atrial myocytes [31]. These observations suggest that activation of the junctional RyRs leads to Ca^{2+} sparks that have the capacity to trigger opening of non-junctional RyR clusters by CICR, but under basal, conditions a firewall composed of mitochondria and Ca^{2+} pumps prevents the inward diffusion of the signal. Another important Ca^{2+} buffer is the myofilaments themselves, which of course contain troponin C. Although the myofilaments are the ultimate target of the Ca^{2+} signal in order to cause contraction, they also contribute to the buffering of Ca^{2+} and hinder movement of the ion within myocytes. Artificially increasing the buffering capacity of the sarcoplasm within atrial myocytes by introducing exogenous Ca^{2+} chelators leads to a sharper definition of the peripheral Ca^{2+} response and diminishes the response from non-junctional RyRs [48]. Diffusion of Ca^{2+} away from the subsarcolemmal initiation sites is therefore critical to trigger propagating CICR deeper in the cell.

Another potential explanation for the lack of centripetal Ca^{2+} waves during EC-coupling under basal conditions is a putative difference in activation or open probability between the junctional and non-junctional RyRs. In support of this notion, it has been demonstrated that the frequencies of spontaneous Ca^{2+} sparks (which can be observed when myocytes are not electrically paced) are significantly greater in the subsarcolemmal region than in the centre of atrial cells [34,57,58] (Fig. 5). It is unclear why such a profound difference should exist between clusters of RyRs within the same cell. However, it does point to a potential functional difference in their activation. The close apposition of junctional RyRs with VOCCs at the peripheral dyadic junctions may underlie the enhanced Ca^{2+} release. It has been demonstrated that the C-terminal tail of L-type VOCCs directly interacts with RyRs [59]. Furthermore, this physical coupling was shown to sensitise RyRs to activation by Ca^{2+}. Obviously, this interaction will not affect the non-junctional RyRs, as they are distant from VOCCs and thus require a higher cytosolic Ca^{2+} rise or larger SR Ca^{2+} load before CICR is initiated [49].

6. How do atrial myocytes evoke global Ca^{2+} transients to enhance contraction?

As described above, for those atrial myocytes without substantial T-tubules, EC-coupling initiates around the periphery of the cells. The expression of powerful Ca^{2+} sequestration pathways in proximity to the zones where EC-coupling initiates disrupts the diffusion of Ca^{2+} from the periphery of the cells, so that the saltatoric centripetal propagation of a Ca^{2+} wave does not occur. However, the function of atrial myocytes is to promote the refilling of ventricles with blood, particularly under times of enhanced physical activity and stress. Therefore, the cells must possess mechanisms that allow Ca^{2+} signals to propagate deeper within the myocytes to engage with troponin C and activate the contractile machinery.

Experimentally, SERCA and mitochondrial Ca^{2+} uptake can be inhibited using specific pharmacological agents. We have observed that inhibition of either process promotes the centripetal movement of Ca^{2+} into the cells, and increases contraction. Physiologically, atrial myocytes do not acutely alter Ca^{2+} sequestration to promote centripetal CICR. Instead, they employ a number of different strategies to ensure that the subsarcolemmal Ca^{2+} rise is able to activate the non-junctional RyRs. In particular, atrial myocytes enhance the trigger Ca^{2+} influx through VOCCs, elevate the Ca^{2+} content of the SR and increase the sensitivity of RyRs to CICR. Each of these three manoeuvres is sufficient by themselves to promote the centripetal propagation of Ca^{2+} into the cells and thereby enhance contraction [31,49]. In combination, these manoeuvres lead to fully global Ca^{2+} signals within atrial myocytes.

A key regulation of the force of cardiac contraction occurs via the sympathetic nervous system, which releases norepinephrine to activate β-adrenergic receptors on the sarcolemma of myocytes [9]. This consequently leads to the activation of adenylyl cyclase, production of cAMP and stimulation of protein kinase A (PKA) activity. PKA has a number of putative targets that promote increased Ca^{2+} signals. For example, PKA can phosphorylate VOCCs, leading to an increase in mean channel open time and/or channel opening probability, thereby enhancing the trigger Ca^{2+} influx following depolarisation [60]. PKA also phosphorylates phospholamban, and possibly sarcolipin, proteins expressed on the SR that interact physically with each other and inhibit Ca^{2+} transport via SERCA [61]. It has been suggested that phosphorylation by PKA dissociates

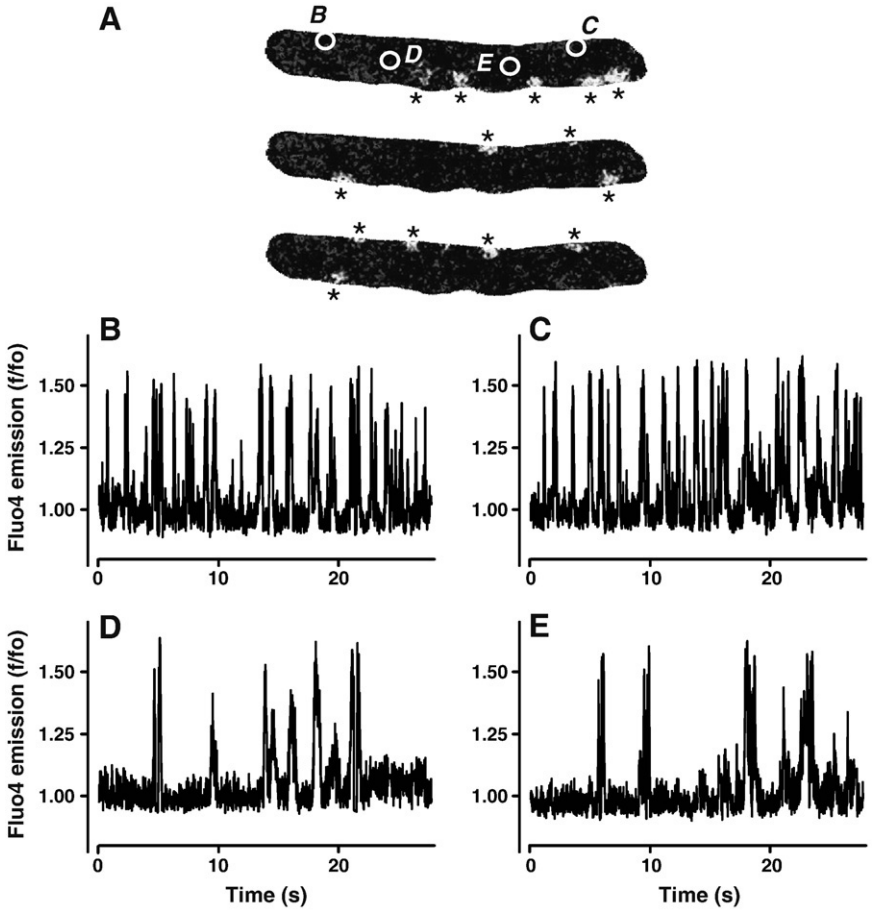

Fig. 5. Increased Ca^{2+} spark activity at the periphery of an atrial myocyte. Panel A shows three images of a single fluo4-loaded rat atrial myocyte that was unpaced. The asterisks illustrate the position of Ca^{2+} sparks around the periphery of the cell. The white circles labelled B–E indicate the cellular regions in which fluo4 fluorescence was sampled to derive the traces in panels B–E. Two peripheral (B and C) and two central (D and E) regions were analysed. The spontaneous Ca^{2+} signals in the central regions were largely Ca^{2+} waves that initiated at the periphery and propagated across the myocyte. In contrast, the periphery of the cell displayed frequent Ca^{2+} sparks that were spatially distinct.

phospholamban/sarcolipin complex from the SERCA pumps and consequently increases Ca^{2+} sequestration. In turn, this leads to an increase in the concentration of Ca^{2+} within the SR lumen. The "gain" of CICR (i.e. the ratio of the resultant Ca^{2+} signal compared with the trigger Ca^{2+} concentration) is steeply dependent on SR Ca^{2+} content [54,62–64]. Evidence from ventricular myocytes suggests that the sensitising effect of luminal Ca^{2+} occurs partly through the Ca^{2+}-binding protein calsequestrin, which reversibly oligomerises in the presence of Ca^{2+} and allosterically regulates RyR opening [65–67]. Calsequestrin is the major Ca^{2+}-binding protein within the SR. It can exist in mono- and oligo-meric forms that have different functions. The oligomeric form is basically a Ca^{2+} buffer that does not interact with RyRs. However, when RyRs open and SR luminal Ca^{2+} declines, Ca^{2+} unbinds from calsequestrin and the oligomeric protein dissociates. Atrial myocytes also express calsequestrin, and it may have a similar function in sensitising Ca^{2+} release (see below). Sympathetic stimulation of the heart therefore engages several molecular mechanisms to generate a positive inotropic effect.

When a β-adrenergic agonist is applied to atrial myocytes, there is a rapid change in the amplitude and spatial properties of the Ca^{2+} signals during EC-coupling. Essentially, the pattern of Ca^{2+} signalling acutely changes from being a peripheral-only elevation of Ca^{2+} to a global response in which the non-junctional RyRs participate [31]. A similar effect can be achieved by directly perfusing atrial myocytes with cAMP via a patch pipette [45]. Concomitant with the change in centripetal propagation of the Ca^{2+} signal, there is a significant increase in contraction [31]. Hormones can thus control atrial contraction by modulating the spatial distribution of the Ca^{2+} signal (in addition to any effect they may have on the sensitivity of the myofilaments to Ca^{2+}). Thus, atrial myocytes are an exemplar of how the simultaneous action of multiple Ca^{2+} transport systems dictates cellular function. The non-junctional myocytes within atrial myocytes therefore constitute a positive inotropic reserve, which can be recruited when stronger cellular contraction is required.

Although RyRs comprise the major Ca^{2+} release mechanism in cardiac myocytes, they are not the only route by which Ca^{2+} can be released from internal stores. An accumulating body of evidence has demonstrated that inositol 1,4,5-trisphosphate receptors ($InsP_3Rs$) are expressed throughout the heart, including within contractile myocytes [68–70]. Ca^{2+} release from $InsP_3Rs$ is triggered in response to stimulation of myocytes with hormones that activate phospholipase C, such as the potent vasoconstrictive peptide hormone endothelin-1. It has been estimated that $InsP_3Rs$ are ~100-fold less abundant than RyRs within the heart [68]. However, they appear to be able to subtly modulate cardiac functions. In particular, $InsP_3Rs$ can regulate gene transcription and stimulate positive inotropy [71–74]. For atrial myocytes, $InsP_3R$ activation enhances the centripetal propagation of atrial Ca^{2+} signals and contraction [31,75]. Pharmacological or genetic ablation of $InsP_3R$ function prevents the positive inotropic effect of agonists such as endothelin-1 on atrial myocytes [71,76].

In contrast to the physiological action of $InsP_3Rs$ as a positively inotropic Ca^{2+} source, $InsP_3R$ activation has also been shown to cause spontaneous Ca^{2+} signals in many studies [74,76,77]. In particular, activation of $InsP_3Rs$ can cause Ca^{2+} signals to arise during the normally quiescent diastolic phase of the cardiac cycle. The generation

of spontaneous Ca^{2+} signals within myocytes has the possibility to perturb the normal beating of myocytes and the propagation of depolarisation throughout the heart. For reasons that are presently not understood, atrial myocytes express substantially more $InsP_3Rs$ than ventricular cells [57,71]. This makes atrial myocytes particularly prone to the potential dysrhythmic effects of $InsP_3R$ activity [76]. It has been reported that $InsP_3R$ expression increases during atrial fibrillation [78]. Furthermore, a pharmacological blocker of $InsP_3R$ function has been demonstrated to reduce atrial fibrillation [78,79]. An example of arrhythmic Ca^{2+} signals caused by $InsP_3R$ activation within an atrial myocyte is depicted in Fig. 6. Perfusion of cells with a membrane-permeant analogue of $InsP_3$ causes both an increase in the amplitude of the systolic Ca^{2+} signals and a concomitant development of spontaneous Ca^{2+} signals.

Ventricular myocytes normally express fewer $InsP_3Rs$ than atrial cells, and are therefore less prone to $InsP_3$-induced perturbation of Ca^{2+} signalling [76]. However, the expression of $InsP_3Rs$ within ventricular myocytes is increased during heart failure [80] and hypertrophic growth of the heart [81,82]. In which case, ventricular myocytes become more like atrial cells in respect of being more responsive to $InsP_3R$ activation, and display greater hormone-induced dysrhythmia [83].

7. Biochemical differences between atrial and ventricular myocytes

In the discussion above, it was noted that the spatial pattern of Ca^{2+} signalling during EC-coupling was different between atrial and ventricular myocytes. A principal reason for this is the lack of T-tubules within atrial cells. However, there are substantial biochemical differences between the cell types that also impact on their Ca^{2+} signals and contractility. In particular, they have different expression levels of key proteins involved in Ca^{2+} signalling and homeostasis. Expression of SERCA2a, for example, is significantly more abundant in atrial cells compared to ventricular myocytes [84], and the atrium consequently has a much greater Ca^{2+} pumping capacity. It is likely that the enhanced SERCA activity accounts for the lower systolic Ca^{2+} transients and more rapid relaxation of atrial myocytes compared to their ventricular counterparts [84,85]. Indeed, altering the expression of SERCA, or its activity, can modulate their rate of relaxation consistent with the major role of SR Ca^{2+} accumulation in terminating contraction [86].

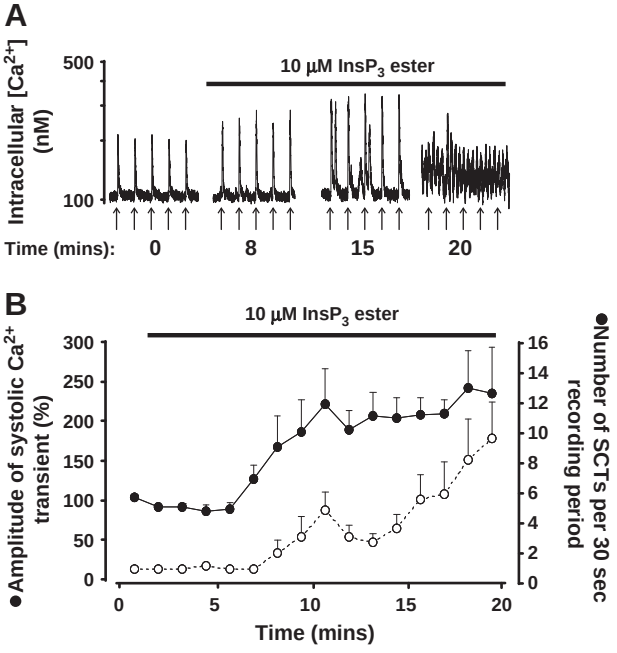

Fig. 6. $InsP_3$ is a positive inotropic and pro-arrhythmic factor in atrial myocytes. The photometry traces in A shows indo-1 signals from single electrically-paced atrial myocyte. The vertical arrows indicate the times at which the cells were electrically paced. The myocyte was superfused with 10 μM $InsP_3$ ester after the steady-state pacing condition had been established. Panel B depicts the effect of continuous stimulation of indo-1-loaded atrial myocytes with $InsP_3$ ester (10 μM) on systolic Ca^{2+} signals and the frequency of spontaneous calcium transients. The data points show the normalised systolic Ca^{2+} transient amplitude monitored using photometry (data capture rate 2 ms). Data were obtained by averaging the peak systolic signal over 30-second periods and sampling at 90-second intervals. The spontaneous Ca^{2+} transient (SCT) frequency was calculated by counting the number of SCTs apparent in the photometry traces during the 30-second intervals when systolic Ca^{2+} was monitored. The symbols indicate mean ± S.E.M ($n = 5$). Reproduced with permission from Ref. [75].

In addition to SERCA, atrial myocytes have a different expression level of several other proteins involved in Ca^{2+} signalling. For example, calsequestrin, junctin and triadin are expressed at lower levels in atrial cells [84]. These proteins form a complex that tunes the activity of RyRs by both direct and indirect interactions. The stoichiometry of these proteins is critical for the fidelity of EC-coupling. Either up- or down-regulation of triadin, for example, can evoke pro-arrhythmic spontaneous Ca^{2+} release. Triadin is responsible for anchoring calsequestrin to RyRs in the junctional cisterns of the SR. Reduction of triadin expression, as occurs during heart failure, causes calsequestrin to be found diffusely within the SR. The calsequestrin monomers bind to RyRs (via triadin) and inhibit channel activity. This is an important component of the mechanisms that terminate Ca^{2+} release during each heartbeat. Mutations in calsequestrin, and concomitant alterations in Ca^{2+} storage/RyR regulation, underlie a condition known as catecholaminergic polymorphic ventricular tachycardia, a life-threatening condition in which stress or exercise can lead to ventricular fibrillation. The distribution of calsequestrin appears to differ between atrial and ventricular myocytes, as might be expected from their distinctive spatial patterns of EC-coupling. In rat ventricular myocytes, the bulk of calsequestrin localises with the I-band indicating that it is largely present within the junctional SR closely apposed to the T-tubules. For rat atrial myocytes, calsequestrin appears more widely distributed. Some calsequestrin is evident along Z-tubules (therefore also the I-band), but in addition the protein is substantially evident in corbular SR deep within the cells, and at the peripheral junctions close to the sarcolemma [87]. The pattern of calsequestrin distribution in rat atrial myocytes appears to support the concept of peripheral initiation of EC-coupling, followed by recruitment of Ca^{2+} stores (e.g. Z-tubules and corbular SR) deeper within the cells.

In ventricular myocytes, SERCA activity is substantially regulated by phospholamban, a 52 amino acid protein that reversibly inhibits Ca^{2+} transport by changing from pentemeric aggregates (when phosphorylated) and inhibitory monomeric forms. However, atrial cells utilise both phospholamban and a smaller (31 amino acid) proteolipid called sarcolipin, to regulate SERCA activity. In this respect, atrial myocytes are similar to skeletal muscle, which also abundantly expresses sarcolipin [88]. In addition to direct inhibition of SERCA, sarcolipin has been suggested to inhibit the polymerisation of phospholamban, and therefore give super-inhibition of SERCA. The expression of atrial sarcolipin is decreased in conditions such as mitral valve disease, atrial fibrillation and hypertrophy [89]. The increased Ca^{2+} pumping that results from sarcolipin downregulation could predispose atrial myocytes to pro-arrhythmic spontaneous Ca^{2+} release.

8. T-tubules within atrial myocytes

The discussion above was largely based on observations from rodent atrial myocytes that do not possess extensive T-tubular systems. The bulk of studies that have examined the spatial properties of atrial myocyte Ca^{2+} signalling have concluded that Ca^{2+} signals rapidly initiated in the periphery of the cells, and have a variable ability to propagate into the cells' interior [31,48]. Indeed, studies in which atrial myocytes were given brief depolarising stimuli, and then

rapidly repolarised, have demonstrated that the peripheral RyRs are essentially under the control of membrane potential (via activation of VOCCs), whereas the non-junctional RyRs are more autonomous (due to on-going CICR) once activated [43,48]. However, some studies have demonstrated Ca^{2+} release within central regions of atrial cells that is synchronous with the peripheral Ca^{2+} response [25,45,90–92] (Fig. 7). The reason for such fast central Ca^{2+} release in atrial myocytes is the presence of T-tubules [10,25,38,39,92]. The degree of T-tubule expression appears to be highly variable between individual atrial myocytes. The majority of rat atrial myocytes, for example, do not possess T-tubules at all. Those atrial myocytes with T-tubules typically have a few limited projections of the sarcolemma [90]. Only a minority of rat atrial cells appear to express a convoluted T-tubule system with a density similar to that seen in ventricular myocytes [25]. However, larger mammals, such as sheep and dog, express T-tubule systems that are reminiscent of ventricular myocytes [91–93], albeit sometimes with large cellular regions devoid of T-tubules.

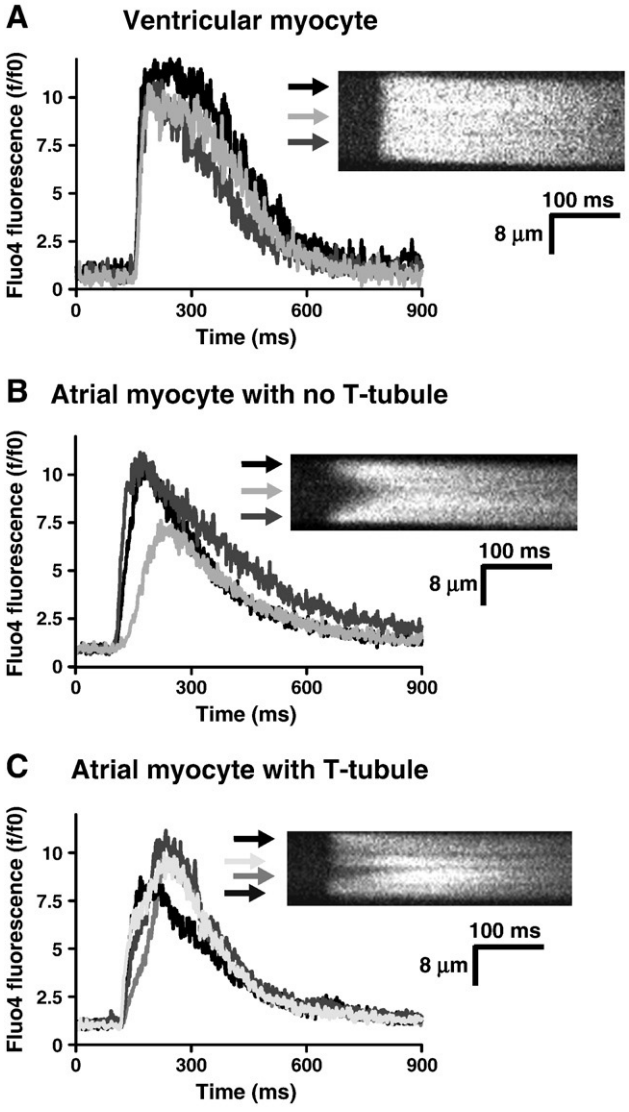

Fig. 7. Ca^{2+} transients in ventricular and atrial myocytes with or without T-tubules. Panels A–C depict the temporal and spatial properties of single depolarisation-evoked Ca^{2+} transients in: A, control ventricular myocyte; B, atrial myocyte without T-tubule system; C, atrial myocyte with an internal initiation site (i.e. with T-tubules). The traces were obtained by sampling fluo-4 intensity along the line-scan images, as depicted by the correspondingly coloured arrows. The data are representative of >10 cells for each condition, sampled from at least three independent preparations. Reproduced with permission from Ref. [25].

Although it has not been extensively demonstrated, it is believed that human atrial myocytes may also be tubulated [29,94].

Clearly, there seems to be a striking difference in the degree of T-tubule expression between mammalian species, and even between cells from the same atrial chamber. Large mammals presumably express an extensive atrial T-tubule system to allow those myocytes to function in a similar manner to ventricular cells. The more variable degree of T-tubule expression observed in rodents is more puzzling, and why these atrial myocytes possess T-tubules at all is not entirely clear. One plausible explanation comes from observations that T-tubule expression correlates with atrial myocyte diameter [25,90]. Since T-tubules penetrate deep into the cells, they can activate CICR within the cell centre and thereby increase the speed at which Ca^{2+} reaches the myofilaments. This would give rise to more synchronous contraction of the muscle when the cells are larger. In addition, by acting as a conduit for extracellular Ca^{2+}, T-tubules enhance SR Ca^{2+} content and speed SR refilling [25]. These effects could lead to more homogenous Ca^{2+} transients within individual myocytes.

However, the density and morphology of T-tubules in rodents do not only differ between ventricular and atrial myocytes, but can also vary in extent between cells of the right and left atrial chambers. Furthermore, within the different atrial chambers, the density and distribution of T-tubules vary from cell to cell [25,39,90,95]. The inconsistency in T-tubule expression, density and morphology in atrial cells is puzzling because it could cause substantial variability in the response of adjacent cells within the working atria. Non-uniformity in contraction of neighbouring cells has been shown to promote arrhythmias [96].

9. The spatial properties of Ca^{2+} signalling in atrial myocytes are relevant to other non-T-tubulated cardiac cells and heart failure

The atrial myocyte Ca^{2+} signalling paradigm of peripheral initiation and centripetal propagation discussed above is also relevant to other tissues where excitation only occurs at the periphery of the cells. For example, cardiac Purkinje cells, which underlie the conduction of action potentials into the mass of ventricular myocytes, display patterns of Ca^{2+} signalling similar to those observed in atrial cells [97,98]. Purkinje cells are non-contractile myocytes solely responsible for the transmission of electrical signals [99]. Like atrial cells, Purkinje have a variable expression of T-tubules [100], but similarly express RyRs in regulated striated patterns throughout their volume [97,101]. Just as with atrial myocytes, Ca^{2+} signals in Purkinje cells initiate in subsarcolemmal locations and show a weak centripetal propagation into the cell interior. Increasing the concentration of extracellular Ca^{2+} or application of a β-adrenergic agonist promotes the response of non-junctional RyRs, but the pattern of peripheral to central propagation is maintained [97]. The physiological consequences of modulating the centripetal propagation of Ca^{2+} within Purkinje cells are not known.

Sinoatrial node cells are another cardiac tissue that does not express extensive T-tubules, and where coupling of VOCCs and RyRs occurs at the periphery of the cells [10]. These cells are responsible for the action potentials that activate contraction of the heart, and display spontaneous electrical depolarisations during late diastole. The mechanisms underlying the spontaneous firing of the sinoatrial node cells have been intensely studied, and two major, non-exclusive, models have been proposed based on membrane potential oscillations or spontaneous Ca^{2+} release events [102]. A time-dependent hyperpolarisation-activated current (commonly suggested to arise from hyperpolarisation-activated cyclic nucleotide-gate channels; I_f) has been shown to drive cells towards the threshold for triggering an action potential. On the other hand, spontaneous Ca^{2+} release events (i.e. Ca^{2+} sparks) occurring in the late diastolic stage have been shown to cause progressive depolarisation by activating NCX [103]. The latter model (often referred to as the "Ca^{2+} clock") has some

resemblance to what happens during pro-arrhythmogenic Ca^{2+} signalling in atrial myocytes, or in the triggering of secondary atrial pacemaking centres (which can fire action potentials to control the heart in the absence of sinoatrial node activity) [104]. In these situations, spontaneous Ca^{2+} release events occur during the normal quiescent period, and if they occur with sufficient frequency they can drive the cell to trigger an action potential [75,105]. Similar to sinoatrial node cells [106], the spontaneous Ca^{2+} signals in atrial myocytes only affect membrane potential if they occur in the sub-sarcolemmal region [105]. Therefore, although sinoatrial node cells and atrial myocytes differ in many biochemical aspects, their lack of T-tubules means that both cell types exhibit peripheral couplings that can respond to and cause action potentials. In the case of sinoatrial node cells and secondary atrial pacemakers, the triggering of subsarcolemmal Ca^{2+} sparks appears to be part of the physiological mechanisms to produce rhythmic heart beats [107]. However, the same activity in the general population of atrial myocytes would produce pro-arrhythmic effects.

The atrial Ca^{2+} signalling paradigm is also relevant to some pathological cardiac conditions. For example, the density of T-tubules in ventricular myocytes decreases dramatically following myocyte damage or heart failure [108–110]. Consequently, Ca^{2+} signals do not arise synchronously throughout ventricular cells, as there are cellular locations where EC-coupling fails to initiate [111,112]. A marked reduction in T-tubule density leads to non-simultaneous recruitment of the Ca^{2+} sparks sites within a ventricular myocyte, which decreases the amplitude and/or rate of rise of the Ca^{2+} signal and diminishes contraction [29,111,113]. In those atrial myocytes that do express T-tubules, it has been demonstrated that the tubules are similarly labile, and can be lost during conditions such as atrial fibrillation [91] or heart failure [92]. If T-tubules are progressively lost, the Ca^{2+} signals within ventricular and tubulated atrial myocytes begin to resemble that of non-tubulated rat atrial cells described above, with a peripheral-only initiation of EC-coupling. A similar effect has been shown using chemically-induced detubulation methods [34] or by growing ventricular cells in long-term culture (where the lack of electrical pacing causes loss of T-tubules) [114,115].

In the same way that physiological atrial Ca^{2+} signals can be boosted by hormonal agonists, the Ca^{2+} signals within detubulated cells can be substantially rescued by stimulation with positive inotropes. For example, application of β-adrenergic stimuli promoted the centripetal propagation of Ca^{2+} within detubulated ventricular cells and restored the kinetics and amplitude of the Ca^{2+} signal [25,113]. It therefore appears that when ventricular and atrial myocytes deteriorate during heart failure, the loss of T-tubules makes their pattern of Ca^{2+} signalling become increasingly like that in non-tubulated atrial cells. They turn out to be dependent on CICR to propagate Ca^{2+} signals away from the peripheral sites where EC-coupling is initiated, and the successful centripetal movement of Ca^{2+} can be reliant on the presence of positive inotropes.

T-tubule development occurs after birth in mammals, with a progressive increase in the density and complexity of sarcolemmal invaginations [116,117]. Neonatal ventricular myocytes are therefore not the same as their adult counterparts. Neonatal cells show some striated protein/organelle distribution, but are still far from being fully organised (Fig. 3C). As with atrial myocytes, the lack of T-tubules in neonatal cells means that RyRs and VOCCs co-localise around the periphery of the cells (Fig. 3C). Consequently, neonatal myocytes display Ca^{2+} signals that are similar to the responses seen in non-tubulated atrial myocytes; they originate at the subsarcolemmal sites and show a diminishing centripetal movement [118,119]. Indeed, many of the features of neonatal cell responses are just like those in non-tubulated atrial cell signals. For example, when stimulated with the RyR agonist caffeine, neonatal cells give homogenous Ca^{2+} signals, indicating that they have a global expression of RyRs and replete Ca^{2+} stores. But the bulk of RyRs deep within the myocytes do not generally participate in EC-coupling. Furthermore, similar to the situation in non-tubulated atrial cells, spontaneous Ca^{2+} sparks also initiate predominantly in subsarcolemmal regions of neonatal myocytes [118]. Since the biochemical properties of RyRs in neonatal and adult ventricular myocytes are functionally equivalent [119], the differences in the spatial pattern of Ca^{2+} signalling can largely be ascribed to the lack of T-tubules. The physiology of neonatal cells is also different from adult tissue because the SR is less well developed and has a smaller Ca^{2+} uptake capacity. Furthermore, neonatal cells express lower levels of L-type VOCCs and RyRs [119].

It is interesting to note that extensive expression of T-tubules is actually a feature of mammalian cells. Ventricular myocytes from non-mammalian vertebrates do not possess these structures. Characterisation of Ca^{2+} signalling in ventricular myocytes from animals such as fish [120] has demonstrated that their responses resemble those described above for mammalian atrial cells. As with mammalian atrial myocytes, the non-mammalian ventricular cells largely form junctional couplings of VOCCs and RyRs around the cellular periphery [121,122]. Peripheral initiation and centripetal propagation are therefore the most commonly observed cardiac Ca^{2+} signalling paradigm within biology. It occurs within the majority of mammalian cardiac cell types (i.e. atrial myocytes, neonatal myocytes, Purkinje cells and SA node cells), is the sole mechanism underlying EC-coupling in other animals and is the process that cardiac cells must rely on during pathologically-evoked changes in their ultrastructure.

10. Conclusion

In summary, it is evident that atrial myocytes employ spatially complex Ca^{2+} signals to regulate their contractile function. For reasons that are not fully understood, there is a substantial degree of variation in the expression of T-tubules in atrial myocytes. This occurs between atrial cells of different animals, between right and left atrial chambers and also between myocytes within the same chamber. The most plausible reason for T-tubule expression in atrial myocytes is to enhance the coordination of Ca^{2+} signals during EC-coupling. However, why this should occur in some species and not others is unclear. The correlation between atrial myocyte size and the presence of T-tubules may account for these observations, in that larger cells cannot solely rely on diffusion and CICR. This notion implies that atrial myocytes possess a mechanism to that can promote the expression of T-tubules when the cells are determined to be a particular size. In non-tubulated atrial myocytes, the Ca^{2+} response that occurs following depolarisation initiates at the periphery of the cells. The success or failure of this subsarcolemmal Ca^{2+} signal to penetrate into the centre of the cells depends substantially on the sensitivity of the non-junctional RyRs, and their ability to perceive a triggering signal in the face of Ca^{2+} buffering and mitochondrial/SERCA activity. Positive inotropic stimuli enhance the centripetal propagation of the Ca^{2+} signal using mechanisms that enhance CICR. Negative inotropes (e.g. acetylcholine and adenosine), on the other hand, would be expected to reduce the centripetal propagation of the Ca^{2+} signals, and sharpen the subsarcolemmal ring of Ca^{2+}. There is a roughly linear relationship between the inward movement of Ca^{2+} and atrial myocyte shortening. The extent of centripetal propagation of the Ca^{2+} signal therefore provides an analog control over cellular contraction. The spatial continuum of Ca^{2+} signalling from peripherally-localised to global responses provides a mechanism by which atrial myocytes can precisely regulate their contribution to blood pumping in the heart. The Ca^{2+} signalling paradigm of peripheral initiation and centripetal propagation is arguably the most commonly encountered EC-coupling mechanism in mammalian cardiac tissues. It applies to cardiac cells that do not express T-tubules, and those that become de-tubulated due to ageing or pathology. Furthermore, it appears to be the principal mechanism underlying contraction of non-mammalian myocytes.

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